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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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☒ *Additional inventors are being named on the 1 separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

MRACAXBCATs AS MODIFIERS OF THE RAC, AXIN, AND BETA-CATENIN PATHWAYS AND METHODS OF USE

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☐ Application Data Sheet. See 37 CFR 1.76

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Respectfully submitted,

SIGNATURE _____ Date 11/25/02

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PROVISIONAL APPLICATION COVER SHEET

Additional Page

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Docket Number	EX02-136P

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EX02-136P

MRACAXBCATs AS MODIFIERS OF THE RAC, AXIN, AND BETA-CATENIN PATHWAYS AND METHODS OF USE

BACKGROUND OF THE INVENTION

Cell movement is an important part of normal developmental and physiological processes (e.g. epiboly, gastrulation and wound healing), and is also important in pathologies such as tumor progression and metastasis, angiogenesis, inflammation and atherosclerosis. The process of cell movement involves alterations of cell-cell and cell-matrix interactions in response to signals, as well as rearrangement of the actin and microtubule cytoskeletons. The small GTPases of the Rho/Rac family interact with a variety of molecules to regulate the processes of cell motility, cell-cell adhesion and cell-matrix adhesion. Cdc42 and Rac are implicated in the formation of filopodia and lamellipodia required for initiating cell movement, and Rho regulates stress fiber and focal adhesion formation. Rho/Rac proteins are effectors of cadherin/catenin-mediated cell-cell adhesion, and function downstream of integrins and growth factor receptors to regulate cytoskeletal changes important for cell adhesion and motility.

There are five members of the Rho/Rac family in the *C. elegans* genome. *rho-1* encodes a protein most similar to human RhoA and RhoC, *cdc-42* encodes an ortholog of human Cdc42, and *ced-10*, *mig-2* and *rac-2* encode Rac-related proteins. *ced-10*, *mig-2* and *rac-2* have partially redundant functions in the control of a number of cell and axonal migrations in the worm, as inactivation of two or all three of these genes causes enhanced migration defects when compared to the single mutants. Furthermore, *ced-10; mig-2* double mutants have gross morphological and movement defects not seen in either single mutant, possibly as a secondary effect of defects in cell migration or movements during morphogenesis. These defects include a completely penetrant uncoordinated phenotype, as well as variably penetrant slow-growth, vulval, withered tail, and sterility defects, none of which are seen in either single mutant.

Beta-catenin is an adherens junction protein. Adherens junctions (AJs; also called the zonula adherens) are critical for the establishment and maintenance of epithelial layers, such as those lining organ surfaces. AJs mediate adhesion between cells,

communicate a signal that neighboring cells are present, and anchor the actin cytoskeleton. In serving these roles, AJs regulate normal cell growth and behavior. At several stages of embryogenesis, wound healing, and tumor cell metastasis, cells form and leave epithelia. This process, which involves the disruption and reestablishment of epithelial cell-cell contacts, may be regulated by the disassembly and assembly of AJs. AJs may also function in the transmission of the 'contact inhibition' signal, which instructs cells to stop dividing once an epithelial sheet is complete.

The AJ is a multiprotein complex assembled around calcium-regulated cell adhesion molecules called cadherins (Peifer, M.(1993) Science 262: 1667-1668). Cadherins are transmembrane proteins: the extracellular domain mediates homotypic adhesion with cadherins on neighboring cells, and the intracellular domain interacts with cytoplasmic proteins that transmit the adhesion signal and anchor the AJ to the actin cytoskeleton. These cytoplasmic proteins include the alpha-, beta-, and gamma-catenins. The beta-catenin protein shares 70% amino acid identity with both plakoglobin, which is found in desmosomes (another type of intracellular junction), and the product of the Drosophila segment polarity gene 'armadillo'. Armadillo is part of a multiprotein AJ complex in Drosophila that also includes some homologs of alpha-catenin and cadherin, and genetic studies indicate that it is required for cell adhesion and cytoskeletal integrity.

Beta-catenin, in addition to its role as a cell adhesion component, also functions as a transcriptional co-activator in the Wnt signaling pathway through its interactions with the family of Tcf and Lef transcription factors (for a review see Polakis, (1999) Current Opinion in Genetics & Development, 9:15–21 and Gat U., et al., (1998) Cell 95:605-614).

Deregulation of beta-catenin signaling is a frequent and early event in the development of a variety of human tumors, including colon cancer, melanoma, ovarian cancer, and prostate cancer. Activation of beta-catenin signaling can occur in tumor cells by loss-of-function mutations in the tumor suppressor genes Axin or APC, as well as by gain-of-function mutations in the oncogene beta-catenin itself. Axin normally functions as a scaffolding protein that binds beta-catenin, APC, and the serine/threonine kinase GSK3-beta. Assembly of this degradation complex allows GSK3-beta to phosphorylate beta-catenin, which leads to beta-catenin ubiquitination and degradation by the

proteasome. In the absence of Axin activity, beta-catenin protein becomes stabilized and accumulates in the nucleus where it acts as a transcriptional co-activator with TCF for the induction of target genes, including the cell cycle regulators cyclin D1 and c-Myc.

The APC gene, which is mutant in adenomatous polyposis of the colon, is a negative regulator of beta-catenin signaling (Korinek, V. et al., (1997) Science 275: 1784-1787; Morin, P. J., et al., (1997) Science 275: 1787-1790). The APC protein normally binds to beta-catenin and, in combination with other proteins (including glycogen synthase kinase-3b and axin, is required for the efficient degradation of b-catenin. The regulation of beta-catenin is critical to the tumor suppressive effect of APC and that this regulation can be circumvented by mutations in either APC or beta-catenin.

While mammals contain only a single beta-catenin gene, *C. elegans* contains three (Korswagen HC, et al., (2000) Nature 406:527-32). Each worm beta-catenin appears to carry out unique functions (Korswagen HC, et al., (2000) Nature 406:527-32, Nartarajan L et al. (2001) Genetics 159: 159-72). Because of the divergence of function in *C. elegans*, it is possible to specifically study beta-catenin role in cell adhesion, which is mediated by the *C. elegans* beta-catenin HMP-2.

The *C. elegans* gene *pry-1* is the structural and functional ortholog of vertebrate Axin (Korswagen HC et al. (2002) Genes Dev. 16:1291-302). PRY-1 is predicted to contain conserved RGS and DIX domains that, in Axin, bind APC and Dishevelled, respectively. Overexpression of the *C. elegans pry-1* gene in zebrafish can fully rescue the mutant phenotype of *masterblind*, the zebrafish Axin1 mutation. *pry-1* loss-of-function mutations produce several phenotypes that appear to result from increased beta-catenin signaling (Gleason JE et al. (2002) Genes Dev. 16:1281-90; Korswagen et al., *supra*).

Mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) is a serine-threonine kinase that activates the c-Jun N-terminal kinase signaling pathway, and may be involved in TNF alpha signaling (Yao, Z., et al (1999) J Biol Chem 274:2118-25; Huang, T. T., et al (2000) Proc Natl Acad Sci U S A 97:1014-9).

KIAA0551 is a protein with high similarity to mitogen-activated protein kinase kinase kinase kinase 6 (MAP4K6) which activates the JUN N terminal kinase (JNK) and p38 MAP kinase pathways.

Misshapen/NIKs-related kinase (MINK) is a member of the germinal center kinase (GCK) family of kinases. MINK activates the cJun N-terminal kinase and the p38 pathways (Dan, I., et al (2000) FEBS Lett 469:19-23).

The ability to manipulate the genomes of model organisms such as *C. elegans* provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and their functions in such model organisms can directly contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example, Dulubova I, et al, J Neurochem 2001 Apr;77(1):229-38; Cai T, et al., Diabetologia 2001 Jan;44(1):81-8; Pasquinelli AE, et al., Nature. 2000 Nov 2;408(6808):37-8; Ivanov IP, et al., EMBO J 2000 Apr 17;19(8):1907-17; Vajo Z et al., Mamm Genome 1999 Oct;10(10):1000-4). For example, a genetic screen can be carried out in an invertebrate model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a "genetic entry point") that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene mutation changes the original phenotype caused by the mutation in the genetic entry point, the gene is identified as a "modifier" involved in the same or overlapping pathway as the genetic entry point. When the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as Rac, axin, and beta-catenin, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

All references cited herein, including patents, patent applications, publications, and sequence information in referenced Genbank identifier numbers, are incorporated herein in their entireties.

SUMMARY OF THE INVENTION

We have discovered genes that modify the Rac, axin, and beta-catenin pathways in *C. elegans*, and identified their human orthologs, hereinafter referred to as

modifiers of Rac, axin, and beta-catenin (MRACAXBCAT). The invention provides methods for utilizing these Rac, axin, and beta-catenin modifier genes and polypeptides to identify MRACAXBCAT-modulating agents that are candidate therapeutic agents that can be used in the treatment of disorders associated with defective or impaired Rac, axin, and beta-catenin function and/or MRACAXBCAT function. Preferred MRACAXBCAT-modulating agents specifically bind to MRACAXBCAT polypeptides and restore Rac, axin, and beta-catenin function. Other preferred MRACAXBCAT-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress MRACAXBCAT gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

MRACAXBCAT modulating agents may be evaluated by any convenient *in vitro* or *in vivo* assay for molecular interaction with an MRACAXBCAT polypeptide or nucleic acid. In one embodiment, candidate MRACAXBCAT modulating agents are tested with an assay system comprising a MRACAXBCAT polypeptide or nucleic acid. Agents that produce a change in the activity of the assay system relative to controls are identified as candidate Rac, axin, and beta-catenin modulating agents. The assay system may be cell-based or cell-free. MRACAXBCAT-modulating agents include MRACAXBCAT related proteins (e.g. dominant negative mutants, and biotherapeutics); MRACAXBCAT-specific antibodies; MRACAXBCAT-specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind to or interact with MRACAXBCAT or compete with MRACAXBCAT binding partner (e.g. by binding to an MRACAXBCAT binding partner). In one specific embodiment, a small molecule modulator is identified using a kinase assay. In specific embodiments, the screening assay system is selected from a binding assay, an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

In another embodiment, candidate Rac, axin, and beta-catenin pathways modulating agents are further tested using a second assay system that detects changes in the Rac, axin, and beta-catenin pathways, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an

agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay system uses non-human animals, including animals predetermined to have a disease or disorder implicating the Rac, axin, and beta-catenin pathways, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

The invention further provides methods for modulating the MRACAXBCAT function and/or the Rac, axin, and beta-catenin pathways in a mammalian cell by contacting the mammalian cell with an agent that specifically binds a MRACAXBCAT polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated the Rac, axin, and beta-catenin pathways.

DETAILED DESCRIPTION OF THE INVENTION

Genetic screens were designed to identify modifiers of the Rac, axin, and beta-catenin pathways in *C. elegans*. For Rac modifiers, the screen was designed to identify modifiers of the Rac signaling pathway that also affect cell migrations in *C. elegans*, where various specific genes were silenced by RNA inhibition (RNAi) in a *ced-10*; *mig-2* double mutant background. For axin modifiers, a reduction of function pry-1 (axin) mutant was used. Various specific genes were silenced by RNA inhibition (RNAi). For beta-catenin modifiers, a weak allele of beta-catenin was used (a homozygous viable mutant of beta-catenin, allele qm39). The hmp-2 (qm-39) strain produces larval worms with a highly penetrant lumpy body phenotype in first stage larval worms (L1s). Various specific genes were silenced by RNA inhibition (RNAi). Methods for using RNAi to silence genes in *C. elegans* are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); WO9932619). Genes causing altered phenotypes in the worms were identified as modifiers of the Rac, axin, and beta-catenin pathways. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, MRACAXBCAT genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of pathologies associated with a defective Rac, axin, and

beta-catenin signaling pathway, such as cancer. Table 1 Example IV) lists the modifiers and their orthologs.

In vitro and in vivo methods of assessing MRACAXBCAT function are provided herein. Modulation of the MRACAXBCAT or their respective binding partners is useful for understanding the association of the Rac, axin, and beta-catenin pathways and their members in normal and disease conditions and for developing diagnostics and therapeutic modalities for Rac, axin, and beta-catenin related pathologies. MRACAXBCAT-modulating agents that act by inhibiting or enhancing MRACAXBCAT expression, directly or indirectly, for example, by affecting an MRACAXBCAT function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. MRACAXBCAT modulating agents are useful in diagnosis, therapy and pharmaceutical development.

Nucleic acids and polypeptides of the invention

Sequences related to MRACAXBCAT nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) or RefSeq number), shown in Table 1 and in the appended sequence listing.

MRACAXBCATs are kinase proteins. The term "MRACAXBCAT polypeptide" refers to a full-length MRACAXBCAT protein or a functionally active fragment or derivative thereof. A "functionally active" MRACAXBCAT fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type MRACAXBCAT protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular substrates, etc. The functional activity of MRACAXBCAT proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan *et al.*, eds., John Wiley & Sons, Inc., Somerset, New Jersey) and as further discussed below. In one embodiment, a functionally active MRACAXBCAT polypeptide is a MRACAXBCAT derivative capable of rescuing defective endogenous MRACAXBCAT activity, such as in cell based or animal assays; the rescuing derivative may be from the same or a different species. For purposes herein, functionally active fragments also include those fragments that comprise one or more

structural domains of an MRACAXBCAT, such as a kinase domain or a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2). Methods for obtaining MRACAXBCAT polypeptides are also further described below. In some embodiments, preferred fragments are functionally active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more preferably 75, and most preferably at least 100 contiguous amino acids a MRACAXBCAT. In further preferred embodiments, the fragment comprises the entire kinase (functionally active) domain.

The term "MRACAXBCAT nucleic acid" refers to a DNA or RNA molecule that encodes a MRACAXBCAT polypeptide. Preferably, the MRACAXBCAT polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and most preferably at least 95% sequence identity with human MRACAXBCAT. Methods of identifying orthlogs are known in the art. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthologs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result retrieves the original query sequence in the reverse BLAST (Huynen MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD et al, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In evolution, when a gene duplication event

follows speciation, a single gene in one species, such as *C.elegans*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, J. Mol. Biol. (1997) 215:403-410) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

A conservative amino acid substitution is one in which an amino acid is substituted for another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute; Smith and Waterman, 1981, J. of Molec.Biol., 147:195-197; Nicholas et al., 1998, "A

Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W.R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of a MRACAXBCAT. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (*e.g.*, Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, Molecular Cloning, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of a MRACAXBCAT under high stringency hybridization conditions that are: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 μg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 μg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.1X SSC and 0.1% SDS (sodium dodecyl sulfate).

In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA;

hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

Alternatively, low stringency conditions can be used that are: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1 x SSC at about 37° C for 1 hour.

Isolation, Production, Expression, and Mis-expression of MRACAXBCAT Nucleic Acids and Polypeptides

MRACAXBCAT nucleic acids and polypeptides, useful for identifying and testing agents that modulate MRACAXBCAT function and for other applications related to the involvement of MRACAXBCAT in the Rac, axin, and beta-catenin pathways. MRACAXBCAT nucleic acids and derivatives and orthologs thereof may be obtained using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods that preserve specific biological activities of these proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes. Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (*e.g.*, generation of fusion proteins). Overexpression of an MRACAXBCAT protein for assays used to assess MRACAXBCAT function, such as involvement in cell cycle regulation or hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (e.g., Higgins SJ and Hames BD (eds.) Protein Expression: A Practical

Approach, Oxford University Press Inc., New York 1999; Stanbury PF et al., Principles of Fermentation Technology, 2nd edition, Elsevier Science, New York, 1995; Doonan S (ed.) Protein Purification Protocols, Humana Press, New Jersey, 1996; Coligan JE et al, Current Protocols in Protein Science (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant MRACAXBCAT is expressed in a cell line known to have defective Rac, axin, and beta-catenin function. The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

The nucleotide sequence encoding an MRACAXBCAT polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native MRACAXBCAT gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, *etc.*); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. An isolated host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

To detect expression of the MRACAXBCAT gene product, the expression vector can comprise a promoter operably linked to an MRACAXBCAT gene nucleic acid, one or more origins of replication, and, one or more selectable markers (*e.g.* thymidine kinase activity, resistance to antibiotics, *etc.*). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the MRACAXBCAT gene product based on the physical or functional properties of the MRACAXBCAT protein in *in vitro* assay systems (*e.g.* immunoassays).

The MRACAXBCAT protein, fragment, or derivative may be optionally expressed as a fusion, or chimeric protein product (i.e. it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection. A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also

be made by protein synthetic techniques, *e.g.* by use of a peptide synthesizer (Hunkapiller *et al.*, Nature (1984) 310:105-111).

Once a recombinant cell that expresses the MRACAXBCAT gene sequence is identified, the gene product can be isolated and purified using standard methods (*e.g.* ion exchange, affinity, and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis). Alternatively, native MRACAXBCAT proteins can be purified from natural sources, by standard methods (*e.g.* immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of MRACAXBCAT or other genes associated with the Rac, axin, and beta-catenin pathways. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (*e.g.* by gene knock-out or blocking expression that would otherwise normally occur).

Genetically modified animals

Animal models that have been genetically modified to alter MRACAXBCAT expression may be used in *in vivo* assays to test for activity of a candidate Rac, axin, and beta-catenin modulating agent, or to further assess the role of MRACAXBCAT in a Rac, axin, and beta-catenin pathways process such as apoptosis or cell proliferation. Preferably, the altered MRACAXBCAT expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal MRACAXBCAT expression. The genetically modified animal may additionally have altered Rac, axin, and beta-catenin expression (e.g. Rac, axin, and beta-catenin knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice or rats), among others. Preferred non-mammalian species include zebrafish, *C. elegans*, and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present

as an extrachromosomal element in a portion of its cells, i.e. mosaic animals (see, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4;870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al., and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050, by Sandford *et al.*; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A.J. *et al.*, A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for microinjection procedures for fish, amphibian eggs and birds see Houdebine and Chourrout, Experientia (1991) 47:897-905; for transgenic rats see Hammer *et al.*, Cell (1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection see, e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson, ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced according to available methods (see Wilmut, I. *et al.* (1997) Nature 385:810-813; and PCT International Publication Nos. WO 97/07668 and WO 97/07669).

In one embodiment, the transgenic animal is a "knock-out" animal having a heterozygous or homozygous alteration in the sequence of an endogenous MRACAXBCAT gene that results in a decrease of MRACAXBCAT function, preferably such that MRACAXBCAT expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out.

Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species. For example, a mouse MRACAXBCAT gene is used to construct a homologous recombination vector suitable for altering an endogenous MRACAXBCAT gene in the mouse genome. Detailed methodologies for homologous recombination in mice are available (see Capecchi, Science (1989) 244:1288-1292; Joyner *et al.*, Nature (1989) 338:153-156). Procedures for the production of non-rodent transgenic mammals and other animals are also available (Houdebine and Chourrout, *supra*; Pursel *et al.*, Science (1989) 244:1281-1288; Simms *et al.*, Bio/Technology (1988) 6:179-183). In a preferred embodiment, knock-out animals, such as mice harboring a knockout of a specific gene, may be used to produce antibodies against the human counterpart of the gene that has been knocked out (Claesson MH et al., (1994) Scan J Immunol 40:257-264; Declerck PJ et al., (1995) J Biol Chem. 270:8397-400).

In another embodiment, the transgenic animal is a "knock-in" animal having an alteration in its genome that results in altered expression (e.g., increased (including ectopic) or decreased expression) of the MRACAXBCAT gene, e.g., by introduction of additional copies of MRACAXBCAT, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the MRACAXBCAT gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

Transgenic nonhuman animals can also be produced that contain selected systems allowing for regulated expression of the transgene. One example of such a system that may be produced is the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.*, PNAS (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase

system is the FLP recombinase system of Saccharomyces cerevisiae (O'Gorman et al. (1991) Science 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment, both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the transgene, and for sequential deletion of vector sequences in the same cell (Sun X et al (2000) Nat Genet 25:83-6).

The genetically modified animals can be used in genetic studies to further elucidate the Rac, axin, and beta-catenin pathways, as animal models of disease and disorders implicating defective Rac, axin, and beta-catenin function, and for *in vivo* testing of candidate therapeutic agents, such as those identified in screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered MRACAXBCAT function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered MRACAXBCAT expression that receive candidate therapeutic agent.

In addition to the above-described genetically modified animals having altered MRACAXBCAT function, animal models having defective Rac, axin, and beta-catenin function (and otherwise normal MRACAXBCAT function), can be used in the methods of the present invention. For example, a Rac, axin, and beta-catenin knockout mouse can be used to assess, *in vivo*, the activity of a candidate Rac, axin, and beta-catenin modulating agent identified in one of the *in vitro* assays described below. Preferably, the candidate Rac, axin, and beta-catenin modulating agent when administered to a model system with cells defective in Rac, axin, and beta-catenin function, produces a detectable phenotypic change in the model system indicating that the Rac, axin, and beta-catenin function is restored.

Modulating Agents

The invention provides methods to identify agents that interact with and/or modulate the function of MRACAXBCAT and/or the Rac, axin, and beta-catenin pathways. Modulating agents identified by the methods are also part of the invention. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the Rac, axin, and beta-catenin pathways, as well as in further analysis of the MRACAXBCAT

protein and its contribution to the Rac, axin, and beta-catenin pathways. Accordingly, the invention also provides methods for modulating the Rac, axin, and beta-catenin pathways comprising the step of specifically modulating MRACAXBCAT activity by administering a MRACAXBCAT-interacting or -modulating agent.

As used herein, an "MRACAXBCAT-modulating agent" is any agent that modulates MRACAXBCAT function, for example, an agent that interacts with MRACAXBCAT to inhibit or enhance MRACAXBCAT activity or otherwise affect normal MRACAXBCAT function. MRACAXBCAT function can be affected at any level, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a preferred embodiment, the MRACAXBCAT - modulating agent specifically modulates the function of the MRACAXBCAT. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the MRACAXBCAT polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the MRACAXBCAT. These phrases also encompasses modulating agents that alter the interaction of the MRACAXBCAT with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of an MRACAXBCAT, or to a protein/binding partner complex, and altering MRACAXBCAT function). In a further preferred embodiment, the MRACAXBCAT- modulating agent is a modulator of the Rac, axin, and beta-catenin pathways (e.g. it restores and/or upregulates Rac, axin, and beta-catenin function) and thus is also a Rac, axin, and beta-catenin-modulating agent.

Preferred MRACAXBCAT-modulating agents include small molecule compounds; MRACAXBCAT-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, 19th edition.

Small molecule modulators

Small molecules are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules, having a molecular weight less than 10,000, preferably less than 5,000, more preferably less than 1,000, and most preferably less than 500. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the MRACAXBCAT protein or may be identified by screening compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can also be identified by screening compound libraries for MRACAXBCAT–modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber SL, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed, optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the Rac, axin, and beta-catenin pathways. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with specific regard to clinical and pharmacological properties. For example, the reagents may be derivatized and re-screened using *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

Protein Modulators

Specific MRACAXBCAT-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the Rac, axin, and beta-catenin pathways and related disorders, as well as in validation assays for other MRACAXBCAT-modulating

agents. In a preferred embodiment, MRACAXBCAT-interacting proteins affect normal MRACAXBCAT function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, MRACAXBCAT-interacting proteins are useful in detecting and providing information about the function of MRACAXBCAT proteins, as is relevant to Rac, axin, and beta-catenin related disorders, such as cancer (e.g., for diagnostic means).

An MRACAXBCAT-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an MRACAXBCAT, such as a member of the MRACAXBCAT pathway that modulates MRACAXBCAT expression, localization, and/or activity. MRACAXBCAT-modulators include dominant negative forms of MRACAXBCAT-interacting proteins and of MRACAXBCAT proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous MRACAXBCAT-interacting proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A Practical Approach, eds. Glover D. & Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL Curr Opin Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29; and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature (2000) 405:837-846; Yates JR 3rd, Trends Genet (2000) 16:5-8).

An MRACAXBCAT-interacting protein may be an exogenous protein, such as an MRACAXBCAT-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory; Harlow and Lane (1999) Using antibodies: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). MRACAXBCAT antibodies are further discussed below.

In preferred embodiments, an MRACAXBCAT-interacting protein specifically binds an MRACAXBCAT protein. In alternative preferred embodiments, an MRACAXBCAT-modulating agent binds an MRACAXBCAT substrate, binding partner, or cofactor.

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Antibodies

In another embodiment, the protein modulator is an MRACAXBCAT specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify MRACAXBCAT modulators. The antibodies can also be used in dissecting the portions of the MRACAXBCAT pathway responsible for various cellular responses and in the general processing and maturation of the MRACAXBCAT.

Antibodies that specifically bind MRACAXBCAT polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of MRACAXBCAT polypeptide, and more preferably, to human MRACAXBCAT. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab').sub.2 fragments, fragments produced by a FAb expression library, anti-idiotypic (anti-Id) antibodies; and epitope-binding fragments of any of the above. Epitopes of MRACAXBCAT which are particularly antigenic can be selected, for example, by routine screening of MRACAXBCAT polypeptides for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Nati. Acad. Sci. U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89; Sutcliffe et al., (1983) Science 219:660-66) to the amino acid sequence of a MRACAXBCAT. Monoclonal antibodies with affinities of 10^8 M^{-1} preferably 10^9 M^{-1} to 10^{10} M^{-1}, or stronger can be made by standard procedures as described (Harlow and Lane, *supra*; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of MRACAXBCAT or substantially purified fragments thereof. If MRACAXBCAT fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of an MRACAXBCAT protein. In a particular embodiment, MRACAXBCAT-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate

immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

The presence of MRACAXBCAT-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding MRACAXBCAT polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

Chimeric antibodies specific to MRACAXBCAT polypeptides can be made that contain different portions from different animal species. For instance, a human immunoglobulin constant region may be linked to a variable region of a murine mAb, such that the antibody derives its biological activity from the human antibody, and its binding specificity from the murine fragment. Chimeric antibodies are produced by splicing together genes that encode the appropriate regions from each species (Morrison et al., Proc. Natl. Acad. Sci. (1984) 81:6851-6855; Neuberger et al., Nature (1984) 312:604-608; Takeda et al., Nature (1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can be generated by grafting complementary-determining regions (CDRs) (Carlos, T. M., J. M. Harlan. 1994. Blood 84:2068-2101) of mouse antibodies into a background of human framework regions and constant regions by recombinant DNA technology (Riechmann LM, et al., 1988 Nature 323: 323-327). Humanized antibodies contain ~10% murine sequences and ~90% human sequences, and thus further reduce or eliminate immunogenicity, while retaining the antibody specificities (Co MS, and Queen C. 1991 Nature 351: 501-501; Morrison SL. 1992 Ann. Rev. Immun. 10:239-265). Humanized antibodies and methods of their production are well-known in the art (U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,762, and 6,180,370).

MRACAXBCAT-specific single chain antibodies which are recombinant, single chain polypeptides formed by linking the heavy and light chain fragments of the Fv regions via an amino acid bridge, can be produced by methods known in the art (U.S. Pat. No. 4,946,778; Bird, Science (1988) 242:423-426; Huston et al., Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883; and Ward et al., Nature (1989) 334:544-546).

Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of

antibodies in phage or similar vectors (Huse et al., Science (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, *supra*).

The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., Int J. Biol Markers (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent emitting lanthanide metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No. 6,086,900).

When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The therapeutically effective dose and dosage regimen is determined by clinical studies. Typically, the amount of antibody administered is in the range of about 0.1 mg/kg –to about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in the range of about 1 mg/ml to about10 mg/ml. Immunotherapeutic methods are further described in the literature (US Pat. No. 5,859,206; WO0073469).

Nucleic Acid Modulators

Other preferred MRACAXBCAT-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit MRACAXBCAT activity. Preferred nucleic acid modulators interfere with the function of the MRACAXBCAT nucleic acid such as DNA replication, transcription, translocation of the MRACAXBCAT RNA to the site of protein translation, translation of protein from the MRACAXBCAT RNA, splicing of the MRACAXBCAT RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the MRACAXBCAT RNA.

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an MRACAXBCAT mRNA to bind to and prevent translation, preferably by binding to the 5′ untranslated region. MRACAXBCAT-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The oligonucleotide can be DNA or RNA or a chimeric mixture or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

In another embodiment, the antisense oligomer is a phosphothioate morpholino oligomer (PMO). PMOs are assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphodiamidate intersubunit linkages. Details of how to make and use PMOs and other antisense oligomers are well known in the art (e.g. see WO99/18193; Probst JC, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev. :7:187-95; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

Alternative preferred MRACAXBCAT nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans*, *Drosophila*, plants, and humans are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619; Elbashir SM, et al., 2001 Nature 411:494-498).

Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a biological pathway. For example, antisense oligomers have been employed as therapeutic moieties in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan JF, *et al*, Current Concepts in Antisense Drug Design, J Med Chem. (1993) 36:1923-1937; Tonkinson JL *et al.*, Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents, Cancer Invest. (1996) 14:54-65). Accordingly, in one aspect of the invention, an MRACAXBCAT-specific nucleic acid modulator is used in an assay to further elucidate the role of the MRACAXBCAT in the Rac, axin, and beta-catenin pathways, and/or its relationship to other members of the pathway. In another aspect of the invention, an MRACAXBCAT-specific antisense oligomer is used as a therapeutic agent for treatment of Rac, axin, and beta-catenin-related disease states.

Assay Systems

The invention provides assay systems and screening methods for identifying specific modulators of MRACAXBCAT activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the MRACAXBCAT nucleic acid or protein. In general, secondary assays further assess the activity of a MRACAXBCAT modulating agent identified by a primary assay and may confirm that the modulating agent affects MRACAXBCAT in a manner relevant to the Rac, axin, and beta-catenin pathways. In some cases, MRACAXBCAT modulators will be directly tested in a secondary assay.

In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an MRACAXBCAT polypeptide or nucleic acid with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. kinase activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-biased activity and the reference activity indicates that the candidate agent modulates MRACAXBCAT activity, and hence the Rac, axin, and beta-catenin pathways. The MRACAXBCAT polypeptide or nucleic acid used in the assay may comprise any of the nucleic acids or polypeptides described above.

Primary Assays

The type of modulator tested generally determines the type of primary assay.

Primary assays for small molecule modulators

For small molecule modulators, screening assays are used to identify candidate modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam GS *et al.*, Curr Opin Chem Biol (1997) 1:384-91 and accompanying references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or

mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including protein-DNA interactions, protein-protein interactions (*e.g.*, receptor-ligand binding), transcriptional activity (*e.g.*, using a reporter gene), enzymatic activity (*e.g.*, via a property of the substrate), activity of second messengers, immunogenicty and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide range of detection methods including fluorescent, radioactive, colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

Cell-based screening assays usually require systems for recombinant expression of MRACAXBCAT and any auxiliary proteins demanded by the particular assay. Appropriate methods for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when MRACAXBCAT-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the MRACAXBCAT protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate MRACAXBCAT-specific binding agents to function as negative effectors in MRACAXBCAT-expressing cells), binding equilibrium constants (usually at least about 10^7 M^{-1}, preferably at least about 10^8 M^{-1}, more preferably at least about 10^9 M^{-1}), and immunogenicity (e.g. ability to elicit MRACAXBCAT specific antibody in a heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of a MRACAXBCAT polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The MRACAXBCAT polypeptide can be full length or a fragment thereof that retains functional MRACAXBCAT activity. The MRACAXBCAT polypeptide may be fused to another

polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The MRACAXBCAT polypeptide is preferably human MRACAXBCAT, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of MRACAXBCAT interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has MRACAXBCAT –specific binding activity, and can be used to assess normal MRACAXBCAT gene function.

Suitable assay formats that may be adapted to screen for MRACAXBCAT modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes PB, Curr Opin Chem Biol (1998) 2:597-603; Sundberg SA, Curr Opin Biotechnol 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved fluorescence, and fluorescence resonance energy transfer. These systems offer means to monitor protein-protein or DNA-protein interactions in which the intensity of the signal emitted from dye-labeled molecules depends upon their interactions with partner molecules (*e.g.*, Selvin PR, Nat Struct Biol (2000) 7:730-4; Fernandes PB, *supra*; Hertzberg RP and Pope AJ, Curr Opin Chem Biol (2000) 4:445-451).

A variety of suitable assay systems may be used to identify candidate MRACAXBCAT and Rac, axin, and beta-catenin pathways modulators (e.g. U.S. Pat. No. 6,165,992 (kinase assays); U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434 (angiogenesis assays), among others). Specific preferred assays are described in more detail below.

Kinase assays. In some preferred embodiments the screening assay detects the ability of the test agent to modulate the kinase activity of a MRACAXBCAT polypeptide. In further embodiments, a cell-free kinase assay system is used to identify a candidate Rac, axin, and beta-catenin modulating agent, and a secondary, cell-based assay, such as an apoptosis or hypoxic induction assay (described below), may be used to further characterize the candidate Rac, axin, and beta-catenin modulating agent. Many different

assays for kinases have been reported in the literature and are well known to those skilled in the art (e.g. U.S. Pat. No. 6,165,992; Zhu et al., Nature Genetics (2000) 26:283-289; and WO0073469). Radioassays, which monitor the transfer of a gamma phosphate are frequently used. For instance, a scintillation assay for p56 (lck) kinase activity monitors the transfer of the gamma phosphate from gamma -^{33}P ATP to a biotinylated peptide substrate; the substrate is captured on a streptavidin coated bead that transmits the signal (Beveridge M *et al.*, J Biomol Screen (2000) 5:205-212). This assay uses the scintillation proximity assay (SPA), in which only radio-ligand bound to receptors tethered to the surface of an SPA bead are detected by the scintillant immobilized within it, allowing binding to be measured without separation of bound from free ligand.

Other assays for protein kinase activity may use antibodies that specifically recognize phosphorylated substrates. For instance, the kinase receptor activation (KIRA) assay measures receptor tyrosine kinase activity by ligand stimulating the intact receptor in cultured cells, then capturing solubilized receptor with specific antibodies and quantifying phosphorylation via phosphotyrosine ELISA (Sadick MD, Dev Biol Stand (1999) 97:121-133).

Another example of antibody based assays for protein kinase activity is TRF (time-resolved fluorometry). This method utilizes europium chelate-labeled anti-phosphotyrosine antibodies to detect phosphate transfer to a polymeric substrate coated onto microtiter plate wells. The amount of phosphorylation is then detected using time-resolved, dissociation-enhanced fluorescence (Braunwalder AF, et al., Anal Biochem 1996 Jul 1;238(2):159-64).

Apoptosis assays. Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of apoptosis (Lazebnik *et al.*, 1994, Nature 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara *et al.*, 1989, J. Exp. Med. 169, 1747). Apoptosis may further be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood 15:4730-41). An apoptosis assay system may comprise a cell that expresses an MRACAXBCAT, and that optionally has defective Rac, axin, and beta-catenin

function (e.g. Rac, axin, and beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the apoptosis assay system and changes in induction of apoptosis relative to controls where no test agent is added, identify candidate Rac, axin, and beta-catenin modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate Rac, axin, and beta-catenin modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether MRACAXBCAT function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express MRACAXBCAT relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the MRACAXBCAT plays a direct role in the apoptotic response. Apoptosis assays are described further in US Pat. No. 6,133,437.

Cell proliferation and cell cycle assays. Cell proliferation may be assayed via bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA. Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino *et al.*, 1986, Int. J. Cancer 38, 369; Campana *et al.*, 1988, J. Immunol. Meth. 107, 79), or by other means.

Cell Proliferation may also be examined using [^{3}H]-thymidine incorporation (Chen, J., 1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73). This assay allows for quantitative characterization of S-phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [^{3}H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter). Another proliferation assay uses the dye Alamar Blue (available from Biosource International), which fluoresces when reduced in living cells and provides an indirect measurement of cell number (Voytik-Harbin SL et al., 1998, In Vitro Cell Dev Biol Anim 34:239-46).

Cell proliferation may also be assayed by colony formation in soft agar (Sambrook et al., Molecular Cloning, Cold Spring Harbor (1989)). For example, cells transformed with

MRACAXBCAT are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW et al. (1986) Int J Radiat Biol Relat Stud Phys Chem Med 49:237-55). Cells transfected with an MRACAXBCAT may be stained with propidium iodide and evaluated in a flow cytometer (available from Becton Dickinson), which indicates accumulation of cells in different stages of the cell cycle.

Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that expresses an MRACAXBCAT, and that optionally has defective Rac, axin, and beta-catenin function (e.g. Rac, axin, and beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the assay system and changes in cell proliferation or cell cycle relative to controls where no test agent is added, identify candidate Rac, axin, and beta-catenin modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate Rac, axin, and beta-catenin modulating agents that is initially identified using another assay system such as a cell-free kinase assay system. A cell proliferation assay may also be used to test whether MRACAXBCAT function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express MRACAXBCAT relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the MRACAXBCAT plays a direct role in cell proliferation or cell cycle.

Angiogenesis. Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an MRACAXBCAT, and that optionally has defective Rac,

axin, and beta-catenin function (e.g. Rac, axin, and beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate Rac, axin, and beta-catenin modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate Rac, axin, and beta-catenin modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether MRACAXBCAT function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express MRACAXBCAT relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the MRACAXBCAT plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others.

Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glyolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with MRACAXBCAT in hypoxic conditions (such as with 0.1% O2, 5% CO2, and balance N2, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an MRACAXBCAT, and that optionally has a mutated Rac, axin, and beta-catenin (e.g. Rac, axin, and beta-catenin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate Rac, axin, and beta-catenin modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate Rac, axin, and beta-catenin modulating agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether MRACAXBCAT function plays a direct role in the hypoxic response. For example, a

hypoxic induction assay may be performed on cells that over- or under-express MRACAXBCAT relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the MRACAXBCAT plays a direct role in hypoxic induction.

Cell adhesion. Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are produced, diluted to 2.5g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2× final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF , and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

High-throughput cell adhesion assays have also been described. In one such assay, small molecule ligands and peptides are bound to the surface of microscope slides using a microarray spotter, intact cells are then contacted with the slides, and unbound cells are washed off. In this assay, not only the binding specificity of the peptides and modulators against cell lines are determined, but also the functional cell signaling of attached cells using immunofluorescence techniques in situ on the microchip is measured (Falsey JR et al., Bioconjug Chem. 2001 May-Jun;12(3):346-53).

Tubulogenesis. Tubulogenesis assays monitor the ability of cultured cells, generally endothelial cells, to form tubular structures on a matrix substrate, which generally simulates the environment of the extracellular matrix. Exemplary substrates include Matrigel™ (Becton Dickinson), an extract of basement membrane proteins containing laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4° C and forms a solid gel at 37° C. Other suitable matrices comprise extracellular components such as collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant, and their ability to form tubules is detected by imaging. Tubules can generally be detected after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Tube formation assays are well known in the art (e.g., Jones MK et al., 1999, Nature Medicine 5:1418-1423). These assays have traditionally involved stimulation with serum or with the growth factors FGF or VEGF. Serum represents an undefined source of growth factors. In a preferred embodiment, the assay is performed with cells cultured in serum free medium, in order to control which process or pathway a candidate agent modulates. Moreover, we have found that different target genes respond differently to stimulation with different pro-angiogenic agents, including inflammatory angiogenic factors such as TNF-alpa. Thus, in a further preferred embodiment, a tubulogenesis assay system comprises testing an MRACAXBCAT's response to a variety of factors, such as FGF, VEGF, phorbol myristate acetate (PMA), TNF-alpha, ephrin, etc.

Cell Migration. An invasion/migration assay (also called a migration assay) tests the ability of cells to overcome a physical barrier and to migrate towards pro-angiogenic signals. Migration assays are known in the art (e.g., Paik JH et al., 2001, J Biol Chem 276:11830-11837). In a typical experimental set-up, cultured endothelial cells are seeded onto a matrix-coated porous lamina, with pore sizes generally smaller than typical cell size. The matrix generally simulates the environment of the extracellular matrix, as described above. The lamina is typically a membrane, such as the transwell polycarbonate membrane (Corning Costar Corporation, Cambridge, MA), and is generally part of an upper chamber that is in fluid contact with a lower chamber

containing pro-angiogenic stimuli. Migration is generally assayed after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Migration is assessed as the number of cells that crossed the lamina, and may be detected by staining cells with hemotoxylin solution (VWR Scientific, South San Francisco, CA), or by any other method for determining cell number. In another exemplary set up, cells are fluorescently labeled and migration is detected using fluorescent readings, for instance using the Falcon HTS FluoroBlok (Becton Dickinson). While some migration is observed in the absence of stimulus, migration is greatly increased in response to pro-angiogenic factors. As described above, a preferred assay system for migration/invasion assays comprises testing an MRACAXBCAT's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

Sprouting assay. A sprouting assay is a three-dimensional *in vitro* angiogenesis assay that uses a cell-number defined spheroid aggregation of endothelial cells ("spheroid"), embedded in a collagen gel-based matrix. The spheroid can serve as a starting point for the sprouting of capillary-like structures by invasion into the extracellular matrix (termed "cell sprouting") and the subsequent formation of complex anastomosing networks (Korff and Augustin, 1999, J Cell Sci 112:3249-58). In an exemplary experimental set-up, spheroids are prepared by pipetting 400 human umbilical vein endothelial cells into individual wells of a nonadhesive 96-well plates to allow overnight spheroidal aggregation (Korff and Augustin: J Cell Biol 143: 1341-52, 1998). Spheroids are harvested and seeded in 900μl of methocel-collagen solution and pipetted into individual wells of a 24 well plate to allow collagen gel polymerization. Test agents are added after 30 min by pipetting 100 μl of 10-fold concentrated working dilution of the test substances on top of the gel. Plates are incubated at 37°C for 24h. Dishes are fixed at the end of the experimental incubation period by addition of paraformaldehyde. Sprouting intensity of endothelial cells can be quantitated by an automated image analysis system to determine the cumulative sprout length per spheroid.

Primary assays for antibody modulators

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the MRACAXBCAT protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, *supra*). The enzyme-linked immunosorbant assay (ELISA) is a preferred method for detecting MRACAXBCAT-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

In some cases, screening assays described for small molecule modulators may also be used to test antibody modulators.

Primary assays for nucleic acid modulators

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance MRACAXBCAT gene expression, preferably mRNA expression. In general, expression analysis comprises comparing MRACAXBCAT expression in like populations of cells (*e.g.*, two pools of cells that endogenously or recombinantly express MRACAXBCAT) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (*e.g.*, using the TaqMan®, PE Applied Biosystems), or microarray analysis may be used to confirm that MRACAXBCAT mRNA expression is reduced in cells treated with the nucleic acid modulator (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the MRACAXBCAT protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra*).

In some cases, screening assays described for small molecule modulators, particularly in assay systems that involve MRACAXBCAT mRNA expression, may also be used to test nucleic acid modulators.

Secondary Assays

Secondary assays may be used to further assess the activity of MRACAXBCAT-modulating agent identified by any of the above methods to confirm that the modulating agent affects MRACAXBCAT in a manner relevant to the Rac, axin, and beta-catenin pathways. As used herein, MRACAXBCAT-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with MRACAXBCAT.

Secondary assays generally compare like populations of cells or animals (*e.g.*, two pools of cells or animals that endogenously or recombinantly express MRACAXBCAT) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate MRACAXBCAT–modulating agent results in changes in the Rac, axin, and beta-catenin pathways in comparison to untreated (or mock- or placebo-treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the Rac, axin, and beta-catenin or interacting pathways.

Cell-based assays

Cell based assays may detect endogenous Rac, axin, and beta-catenin pathways activity or may rely on recombinant expression of Rac, axin, and beta-catenin pathways components. Any of the aforementioned assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any other efficacious means.

Animal Assays

A variety of non-human animal models of normal or defective Rac, axin, and beta-catenin pathways may be used to test candidate MRACAXBCAT modulators. Models for defective Rac, axin, and beta-catenin pathways typically use genetically modified animals that have been engineered to mis-express (*e.g.*, over-express or lack expression in) genes involved in the Rac, axin, and beta-catenin pathways. Assays generally require

systemic delivery of the candidate modulators, such as by oral administration, injection, etc.

In a preferred embodiment, Rac, axin, and beta-catenin pathways activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal Rac, axin, and beta-catenin are used to test the candidate modulator's affect on MRACAXBCAT in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4° C, but rapidly forms a solid gel at 37° C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the MRACAXBCAT. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 - 12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

In another preferred embodiment, the effect of the candidate modulator on MRACAXBCAT is assessed via tumorigenicity assays. Tumor xenograft assays are known in the art (see, e.g., Ogawa K et al., 2000, Oncogene 19:6043-6052). Xenografts are typically implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from *in vitro* culture. The tumors which express the MRACAXBCAT endogenously are injected in the flank, 1×10^5 to 1×10^7 cells per mouse in a volume of 100 µL using a 27gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4%

paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

In another preferred embodiment, tumorogenicity is monitored using a hollow fiber assay, which is described in U.S. Pat No. US 5,698,413. Briefly, the method comprises implanting into a laboratory animal a biocompatible, semi-permeable encapsulation device containing target cells, treating the laboratory animal with a candidate modulating agent, and evaluating the target cells for reaction to the candidate modulator. Implanted cells are generally human cells from a pre-existing tumor or a tumor cell line. After an appropriate period of time, generally around six days, the implanted samples are harvested for evaluation of the candidate modulator. Tumorogenicity and modulator efficacy may be evaluated by assaying the quantity of viable cells present in the macrocapsule, which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate in vitro, etc.

In another preferred embodiment, a tumorogenicity assay use a transgenic animal, usually a mouse, carrying a dominant oncogene or tumor suppressor gene knockout under the control of tissue specific regulatory sequences; these assays are generally referred to as transgenic tumor assays. In a preferred application, tumor development in the transgenic model is well characterized or is controlled. In an exemplary model, the "RIP1-Tag2" transgene, comprising the SV40 large T-antigen oncogene under control of the insulin gene regulatory regions is expressed in pancreatic beta cells and results in islet cell carcinomas (Hanahan D, 1985, Nature 315:115-122; Parangi S et al, 1996, Proc Natl Acad Sci USA 93: 2002-2007; Bergers G et al, 1999, Science 284:808-812). An "angiogenic switch," occurs at approximately five weeks, as normally quiescent capillaries in a subset of hyperproliferative islets become angiogenic. The RIP1-TAG2 mice die by age 14 weeks. Candidate modulators may be administered at a variety of stages, including just prior to the angiogenic switch (e.g., for a model of tumor prevention), during the growth of small tumors (e.g., for a model of intervention), or during the growth of large and/or invasive tumors (e.g., for a model of regression). Tumorogenicity and modulator efficacy can be evaluating life-span extension and/or

beta-catenin pathways and for the identification of subjects having a predisposition to such diseases and disorders.

Various expression analysis methods can be used to diagnose whether MRACAXBCAT expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective Rac, axin, and beta-catenin signaling that express an MRACAXBCAT, are identified as amenable to treatment with an MRACAXBCAT modulating agent. In a preferred application, the Rac, axin, and beta-catenin defective tissue overexpresses an MRACAXBCAT relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial MRACAXBCAT cDNA sequences as probes, can determine whether particular tumors express or overexpress MRACAXBCAT. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of MRACAXBCAT expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

Various other diagnostic methods may be performed, for example, utilizing reagents such as the MRACAXBCAT oligonucleotides, and antibodies directed against an MRACAXBCAT, as described above for: (1) the detection of the presence of MRACAXBCAT gene mutations, or the detection of either over- or under-expression of MRACAXBCAT mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of MRACAXBCAT gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by MRACAXBCAT.

Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease or disorder in a patient that is associated with alterations in MRACAXBCAT expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for MRACAXBCAT expression; c) comparing

suppressed by loss-of-function mutations in the beta-catenin ortholog *bar-1* and the TCF ortholog *pop-1*. The Rvl phenotype can also be generated by gain-of-function mutations in *bar-1*/beta-catenin that eliminate the consensus GSK3-beta phosphorylation sites and are predicted to prevent Axin-mediated degradation of BAR-1.

We designed a genetic screen to identify genes in addition to *bar-1*/beta-catenin and *pop-1*/TCF that act positively in beta-catenin signaling and, when inactivated, suppress the Rvl mutant phenotype of *pry-1*/Axin. The function of individual genes was inactivated by RNAi in *pry-1 (mu38)* L1 larvae, and suppression of the Rvl phenotype was scored as a statistically significant increase in the proportion of larvae that survived to adulthood without rupturing. Suppressor genes were subsequently counterscreened to eliminate those that appeared to suppress the *pry-1* mutant non-specifically, rather than those that specifically functioned in beta-catenin signaling. Suppressor genes that did not block vulva formation in a wildtype background, and that did not suppress the Rvl phenotype of two mutations in genes unrelated to beta-catenin signaling (*lin-1*/Ets and *daf-18*/PTEN) were considered to be specific *pry-1*/Axin suppressors. These suppressor genes, when inactivated, likely suppress beta-catenin's inappropriate transcriptional activation of target genes and, therefore, may be relevant for cancer therapy.

III. C. elegans beta-catenin screen

The identification of mutants that suppress the cell adhesion defect of beta-catenin may lead to unique therapeutic targets that inhibit cell migration or metastasis. *hmp-2* was initially identified in an EMS screen for defects in body elongation during embryonic morphogenesis (see Costa *et al.*, (1998) The Journal of Cell Biology 1998, 141: 297-308). The loss of function allele *hmp-2 (zu364)* exhibits 99% embryonic lethality, with mutant embryos arresting during elongation and abnormal bulges forming on the dorsal side. About 1% of these embryos hatch to form viable lumpy larvae. The reduction of function allele *hmp-2 (qm39)* yields viable larvae with a characteristic lumpy appearance. When grown at 15°C, approximately 92% (SD 3.9) of the L1 larvae show this lumpy phenotype, with the penetrance of the phenotype decreasing as the animals molt and move through successive larval stages. For this screen, *hmp-2 (qm39)* worms were soaked at 15°C in double stranded RNA (dsRNA) at the L4 larval stage and the progeny

were scored as L1 larvae for modification of the adhesion defect. The screen protocol is described below.

1) *hmp-2 (qm39)* animals were bleached and hatched on peptone free agarose plates to produce a synchronous population. Starved L1s were transferred to 10x peptone plates seeded with 750 µl OP50 (25% w/v in TB) and allowed to develop to the *L4* larval stage.
2) dsRNA was dispensed in 6 µl aliquots into 96 well round bottom plates (Nunc #262162). *L4* animals were collected by suspension in M9 buffer, washed 2x with M9 to remove any excess OP50, and dispensed in 2 µl aliquots into the RNA to a total worm density of 75-100 worms per well. As a control, multiple wells contained only RNA resuspension buffer (1x IM buffer).
3) Animals were soaked in dsRNA at 15°C for 24 hours.
4) Following dsRNA soaking, the animals were fed in the wells by addition of 25µl liquid NGM + 3% OP50. The animals were kept at 15°C and allowed to become gravid and lay progeny in the wells, which took approximately 72 hours. Food levels were monitored visually during maturation and more was added as needed.
5) Following maturation, animals from each well were plated onto individual 6cm peptone free agarose plates and placed at 15°C overnight.
6) Animals on each plate were scored visually under the dissecting microscope for modification of the lumpy phenotype. Scoring was performed qualitatively, with an increase in dead embryos scored as enhancement and an increase in wild type appearing animals scored as suppression of the defect.
7) Retests of interesting suppressor candidates followed the same protocol as the primary screen with certain modifications: several retests were performed for each suppressor, retested candidates were encoded so that they could be scored blindly, and retested candidates were scored quantitatively. Each plate was scored by counting 100 total objects. An object was defined as either an embryo or an L1 stage larva. Each object was scored as one of the following: a wildtype appearing animal, a lumpy appearing animal, or an unhatched embryo. Scores were represented as the percentage of wildtype appearing animals relative to all objects scored. Wildtype

animals were defined as L1 larvae with smooth cuticles that did not have any sort of lumpy body morphology.

8) A confirmed suppressor was one that was ≥ 2 standard deviations away from the mean of the controls for at least 3 of the four retest experiments.

IV. Analysis of Table 1

BLAST analysis (Altschul et al., *supra*) was employed to identify Targets from *C. elegans* modifiers. The columns "MRACAXBCAT symbol", and "MRACAXBCAT name aliases " provide a symbol and the known name abbreviations for the Targets, where available, from Genbank. "MRACAXBCAT RefSeq_NA or GI_NA", "MRACAXBCAT GI_AA", "MRACAXBCAT NAME", and "MRACAXBCAT Description" provide the reference DNA sequences for the MRACAXBCATs as available from National Center for Biology Information (NCBI), MRACAXBCAT protein Genbank identifier number (GI#), MRACAXBCAT name, and MRACAXBCAT description, all available from Genbank, respectively. The length of each amino acid is in the "MRACAXBCAT Protein Length" column.

Names and Protein sequences of *C. elegans* modifiers of Rac, axin, and beta-catenin from screen (Examples I, II, and III), are represented in the "Modifier Name" and "Modifier GI_AA" column by GI#, respectively.

TABLE1

MRACAXBCAT Symbol	MRACAXBCAT Name Aliases	MRACAXBCAT RefSeq_NA or GI_NA	MRACAXBCAT GI_aa	MRACAXBCAT name	MRACAXBCAT Description	MRACAXBCAT Protein length	Modifier Name	Modifier GI_aa

ZC2/KIAA0551	ZC2 | KIAA0551 protein | KIAA0551	XM_039796.8	6110350	KIAA0551 protein	Protein with high similarity to mitogen-activated protein kinase kinase kinase kinase 4 (human MAP4K4), which activates the c-Jun N-terminal kinase (human MAPK8) signaling pathway, contains a protein kinase domain and a regulatory citron homology domain	1331	ZC504.4	17569073
MAP4K4	HGK | NIK | FLH21957 | KIAA0687 | HPK/GCK-like kinase | mitogen-activated protein kinase kinase kinase kinase 4 | MAP4K4 | mig-15	NM_004834.2 | NM_145686.1 | NM_145687.1	22035602	mitogen-activated protein kinase kinase kinase kinase 4	Mitogen-activated protein kinase kinase kinase kinase 4, a serine-threonine kinase, activates the c-Jun N-terminal kinase (MAPK8) signaling pathway, does not activate the ERK or p38 (CSBP1) kinase pathways, may be involved in TNF-alpha (TNF) signaling	1166	ZC504.4	17569073
MINK	MINK | Misshapen/ NIK-related kinase	NM_015716.1 | NM_153827.1 | NM_170663.1 | XM_113957.2	7657335	Misshapen/ NIK-related kinase	Misshapen/NIK-related kinase, a member of the germinal center kinase (GCK) family, may activate the cJUN N terminal kinase (JNK) and p38 MAP kinase pathways	1295	ZC504.4	17569073

V. High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled MRACAXBCAT peptide/substrate are added to each well of a 96-well microtiter plate, along with a test agent in a test buffer (10 mM HEPES, 10 mM NaCl, 6 mM magnesium chloride, pH 7.6). Changes in fluorescence polarization, determined by using a Fluorolite FPM-2 Fluorescence Polarization Microtiter System (Dynatech Laboratories, Inc), relative to control values indicates the test compound is a candidate modifier of MRACAXBCAT activity.

VI. High-Throughput In Vitro Binding Assay.

^{33}P-labeled MRACAXBCAT peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-avidin coated assay plate and incubated at 25°C for 1 hour. Biotinylated substrate is then added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS, and counted in a scintillation counter. Test agents that cause a difference in activity relative to control without test agent are identified as candidate Rac, axin, and beta-catenin modulating agents.

VII. Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins, 3×10^6 appropriate recombinant cells containing the MRACAXBCAT proteins are plated on 10-cm dishes and transfected on the following day with expression constructs. The total amount of DNA is kept constant in each transfection by adding empty vector. After 24 h, cells are collected, washed once with phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer containing 50 mM Hepes, pH 7.9, 250 mM NaCl, 20 mM -glycerophosphate, 1 mM sodium orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular debris is removed by centrifugation twice at 15,000 × g for 15 min. The cell lysate is incubated with 25 μl of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

After extensive washing with lysis buffer, proteins bound to the beads are solubilized by boiling in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane and blotted with the indicated antibodies. The reactive bands are visualized with horseradish peroxidase coupled to the appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham Pharmacia Biotech).

VIII. Kinase assay

A purified or partially purified MRACAXBCAT is diluted in a suitable reaction buffer, e.g., 50 mM Hepes, pH 7.5, containing magnesium chloride or manganese chloride (1-20 mM) and a peptide or polypeptide substrate, such as myelin basic protein

or casein (1-10 μg/ml). The final concentration of the kinase is 1-20 nM. The enzyme reaction is conducted in microtiter plates to facilitate optimization of reaction conditions by increasing assay throughput. A 96-well microtiter plate is employed using a final volume 30-100 μl. The reaction is initiated by the addition of ^{33}P-gamma-ATP (0.5 μCi/ml) and incubated for 0.5 to 3 hours at room temperature. Negative controls are provided by the addition of EDTA, which chelates the divalent cation ($Mg2^{+}$ or Mn^{2+}) required for enzymatic activity. Following the incubation, the enzyme reaction is quenched using EDTA. Samples of the reaction are transferred to a 96-well glass fiber filter plate (MultiScreen, Millipore). The filters are subsequently washed with phosphate-buffered saline, dilute phosphoric acid (0.5%) or other suitable medium to remove excess radiolabeled ATP. Scintillation cocktail is added to the filter plate and the incorporated radioactivity is quantitated by scintillation counting (Wallac/Perkin Elmer). Activity is defined by the amount of radioactivity detected following subtraction of the negative control reaction value (EDTA quench).

IX. Expression analysis

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues are obtained from Impath, UC Davis, Clontech, Stratagene, Ardais, Genome Collaborative, and Ambion.

TaqMan analysis is used to assess expression levels of the disclosed genes in various samples.

RNA is extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy kits, following manufacturer's protocols, to a final concentration of 50ng/μl. Single stranded cDNA is then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, CA).

Primers for expression analysis using TaqMan assay (Applied Biosystems, Foster City, CA) are prepared according to the TaqMan protocols, and the following criteria: a) primer pairs are designed to span introns to eliminate genomic contamination, and b)

each primer pair produced only one product. Expression analysis is performed using a 7900HT instrument.

Taqman reactions are carried out following manufacturer's protocols, in 25 µl total volume for 96-well plates and 10 µl total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis is prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data are normalized using 18S rRNA (universally expressed in all tissues and cells).

For each expression analysis, tumor tissue samples are compared with matched normal tissues from the same patient. A gene is considered overexpressed in a tumor when the level of expression of the gene is 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue is not available, a universal pool of cDNA samples is used instead. In these cases, a gene is considered overexpressed in a tumor sample when the difference of expression levels between a tumor sample and the average of all normal samples from the same tissue type is greater than 2 times the standard deviation of all normal samples (i.e., Tumor – average(all normal samples) > 2 x STDEV(all normal samples)).

A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

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WHAT IS CLAIMED IS:

1. A method of identifying a candidate Rac, axin, and beta-catenin pathways modulating agent, said method comprising the steps of:

(a) providing an assay system comprising a MRACAXBCAT polypeptide or nucleic acid;

(b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and

(c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate Rac, axin, and beta-catenin pathways modulating agent.

2. The method of Claim 1 wherein the assay system comprises cultured cells that express the MRACAXBCAT polypeptide.

3. The method of Claim 2 wherein the cultured cells additionally have defective Rac, axin, and beta-catenin function.

4. The method of Claim 1 wherein the assay system includes a screening assay comprising a MRACAXBCAT polypeptide, and the candidate test agent is a small molecule modulator.

5. The method of Claim 4 wherein the assay is a kinase assay.

6. The method of Claim 1 wherein the assay system is selected from the group consisting of an apoptosis assay system, a cell proliferation assay system, an angiogenesis assay system, and a hypoxic induction assay system.

7. The method of Claim 1 wherein the assay system includes a binding assay comprising a MRACAXBCAT polypeptide and the candidate test agent is an antibody.

8. The method of Claim 1 wherein the assay system includes an expression assay comprising a MRACAXBCAT nucleic acid and the candidate test agent is a nucleic acid modulator.

9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.

10. The method of Claim 8 wherein the nucleic acid modulator is a PMO.

11. The method of Claim 1 additionally comprising:

(d) administering the candidate Rac, axin, and beta-catenin pathways modulating agent identified in (c) to a model system comprising cells defective in Rac, axin, and beta-catenin function and, detecting a phenotypic change in the model system that indicates that the Rac, axin, and beta-catenin function is restored.

12. The method of Claim 11 wherein the model system is a mouse model with defective Rac, axin, and beta-catenin function.

13. A method for modulating a Rac, axin, and beta-catenin pathways of a cell comprising contacting a cell defective in Rac, axin, and beta-catenin function with a candidate modulator that specifically binds to a MRACAXBCAT polypeptide, whereby Rac, axin, and beta-catenin function is restored.

14. The method of claim 13 wherein the candidate modulator is administered to a vertebrate animal predetermined to have a disease or disorder resulting from a defect in Rac, axin, and beta-catenin function.

15. The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.

16. The method of Claim 1, comprising the additional steps of:

(d) providing a secondary assay system comprising cultured cells or a non-human animal expressing MRACAXBCAT ,

(e) contacting the secondary assay system with the test agent of (b) or an agent derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system,

wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate Rac, axin, and beta-catenin pathways modulating agent,

and wherein the second assay detects an agent-biased change in the Rac, axin, and beta-catenin pathways.

17. The method of Claim 16 wherein the secondary assay system comprises cultured cells.

18. The method of Claim 16 wherein the secondary assay system comprises a non-human animal.

19. The method of Claim 18 wherein the non-human animal mis-expresses a Rac, axin, and beta-catenin pathways gene.

20. A method of modulating Rac, axin, and beta-catenin pathways in a mammalian cell comprising contacting the cell with an agent that specifically binds a MRACAXBCAT polypeptide or nucleic acid.

21. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the Rac, axin, and beta-catenin pathways.

22. The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

23. A method for diagnosing a disease in a patient comprising:

(a) obtaining a biological sample from the patient;

(b) contacting the sample with a probe for MRACAXBCAT expression;

(c) comparing results from step (b) with a control;

(d) determining whether step (c) indicates a likelihood of disease.

24. The method of claim 23 wherein said disease is cancer.

ABSTRACT OF THE DISCLOSURE

Human MRACAXBCAT genes are identified as modulators of the Rac, axin, and beta-catenin pathways, and thus are therapeutic targets for disorders associated with defective Rac, axin, and beta-catenin function. Methods for identifying modulators of Rac, axin, and beta-catenin, comprising screening for agents that modulate the activity of MRACAXBCAT are provided.

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Nucleic Acid and Polypeptide sequences

>gi|22041067|ref|XM_039796.8| Homo sapiens KIAA0551 protein (KIAA0551), mRNA

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>gi|6110350|gb|AAF03783.1|AF172265_1 Traf2 and NCK interacting kinase, splice variant 2 [Homo sapiens]
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>gi|22035602|ref|NP_004825.2| mitogen-activated protein kinase kinase kinase kinase 4 isoform 1; HPK/GCK-like kinase [Homo sapiens]
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>gi|7657335|ref|NP_056531.1| Misshapen/NIK-related kinase [Homo sapiens]
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